

Interferometric Localization Microscopy

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Abstract: Interference of signal in Fourier space, emitted from single probes, is used to localize it by recording and computing the phase of the fringes. Such system has applications in super resolution localization microscopy.

OCIS codes: (100.6640) Superresolution; (110.0180) Microscopy

1. Introduction

Superresolution techniques, developed to overcome the diffraction limit, gained much interest in the last two decades. The emergence of techniques such as NSOM [1–3], STED [4,5], PALM/STORM [6–8] enabled imaging of biological structures with resolution of tens of nanometers. The basic principle of operation in these techniques did not change significantly over the years. In NSOM scanning of the sample from a close distance, where near field details which are not limited by diffraction can be recorded. STED relies on scanning of the sample by sub-diffraction size spot obtained by depleting the fluorescence probes around that spot, while superresolution microscopy modalities such as PALM and STORM rely on the precise localization of point emitters for their sub-diffraction-limited resolution. The localization precision in these schemes is limited by the signal-to-noise ratio of the collected signal. Specifically, the uncertainty of the location of the probe is inversely proportional to the square root of the number of detected photons [9]. Localization techniques rely on computing the center of the PSF directly or via fitting to a modeled PSF, using the signal intensity. In this work we use the phase of the recorded signal in order to localize the emitting probe to a higher precision than is otherwise feasible.

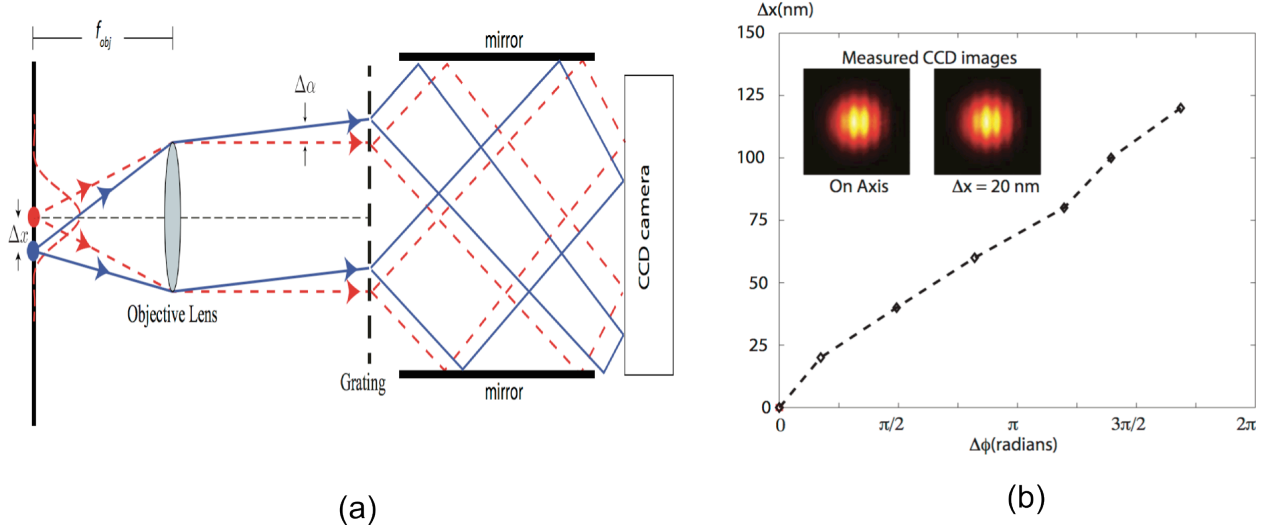


Figure 1 Interferometric localization microscopy system. (a) A spatial shift in the sample plane translates into an angle at the entrance to interferometer in the Fourier plane. The angle induces path length differences between arms and thus phase for the interference fringes. (b) Relation between measured phase and spatial shift.

2. System and Results

The schematic of the optical system can be seen in Figure 1a. A spatial shift of a point emitter in the sample plane translates into an angle in Fourier domain. The signal then passes through a diffraction grating designed to have maximum efficiency in the ± 1 orders. The two orders are reflected from the mirrors and combine onto the camera through a second grating. The optical path length difference between the two interferometer arms induces a phase

shift which can be detected and translated back into a spatial position of the emitting probe. The one dimensional interference pattern at the camera can be written as

$$I(x) = OTF(x) \left(1 + \gamma \cos(\omega x + \phi) \right), \quad (1)$$

where γ is the fringe visibility, ω is the fringe spatial frequency and ϕ is the phase. A scanning mirror was used to change the incident angle of a HeNe laser onto the grating. This angle corresponds to a pre-calibrated spatial shift in the sample plane. The relation between the measured phase and the spatial shift, which corresponds to each angle can be seen in Figure 1b. To establish the ability of the system to localize single emitters with accuracy of 20nm we imaged Au nanospheres with diameter of 100nm (GNP). The GNP were laid on top of a silicon wafer, placed on top of a scanning stage and illuminated with 561nm laser. The reflection from the sample was recorded using an APD, while the GNP appeared as diffraction limited dark spots due to their high absorption. After initial imaging using a scanning method, one GNP was isolated and the illumination beam was directed at its center. The stage was then moved 20nm at a time and 10 frames of the output of the system in for each stage position were recorded Figure 1 (where at this stage we added a second diffraction grating just before the camera). An example of the interference pattern can be seen in Figure 2a. The interference pattern was then rotated to have the fringes along the image y axis, and then summed to form a vector. This vector was then fitted to equation (1), where the OTF was assumed to have a Gaussian shape, and the phase ϕ was found. The results of the stage position vs. the phase averaged over 10 frames are presented in Figure 2b. These results show that the obtained phase value indicate the stage position, i.e. the distance of the nanoparticle from the optical axis.

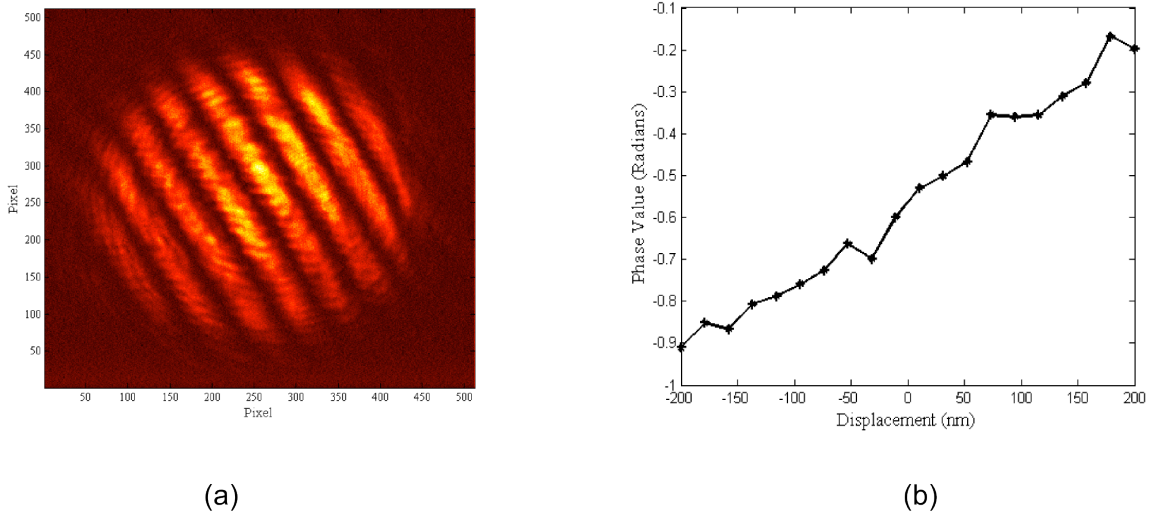


Figure 2 (a) Interference fringes at the output of the grating system. (b) Phase value vs. displacement of the nanoparticles from the optical axis.

3. Conclusions

The presented method can be used in localization microscopy techniques which rely on metal nanoparticles to tag the sample [10–12]. In these techniques, metal nanoparticles are used as the probes rather than fluorescent probes, and the signal share the same bandwidth as the laser source. Scattering or absorption from the nanoparticles is viewed as diffraction limited spots which can be localized. The narrow spectrum allows the use of the grating system where the fringes as clearly visible.

The technique presented here uses Fourier domain phase fitting to localize the nanoparticles to high precision of approximately 20nm. The localization accuracy in such a system is therefore limited by the size of the particles, where the acquisition speed is possible due to the high absorption and scattering of the metal nanoparticles. The use of absorption mode limits the shot noise, due to the high reflectivity of the silicon surface, where even in the middle of the highly absorbing nanoparticles induced PSF, the photon count is high.

4. References

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